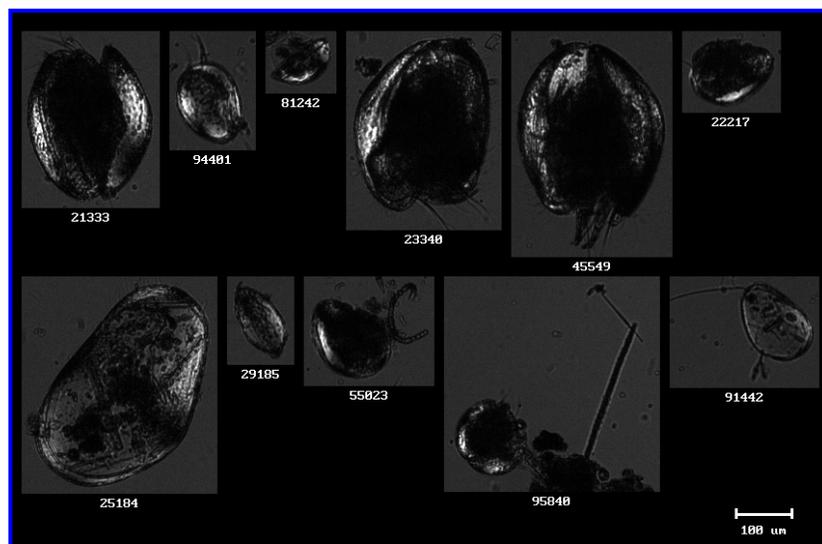


## FlowCAM Early Detection Key to Slowing Spread of Zebra and Quagga Mussels

Clean Lakes, Inc. (CLI) has continued to lead the fight against the spread of zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena rostriformis bugensis*) throughout the past year via our laboratory early detection services utilizing the FlowCAM technology as well as participating in industry wide protocol and laboratory standard development. Our clients have ranged geographically from the West to the Great Lakes and their requirements have ranged analytically from individual location expedited results to season long system wide analysis. The need for timely and accurate early detection of this invasive species has never been more acute than now as evidenced by the continued spread of zebra and quagga mussels in addition to inaccurate laboratory positive identifications spreading unneeded alarm. I will discuss two of CLI's 2010 client needs as well as the steps our laboratory has taken to ensure our early detection services are timely while delivering industry-wide accepted accuracy.

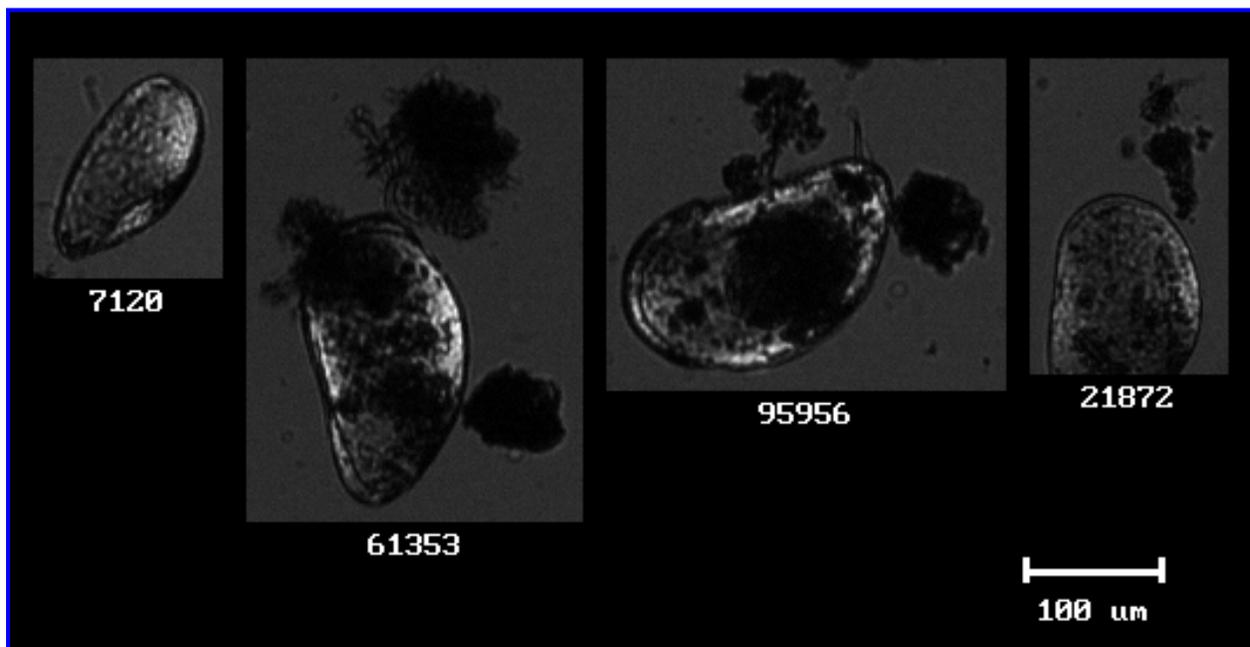
An example of systems wide, full season analysis comes from the Great Lakes region, where CLI assisted a National Scenic Riverway (name withheld due to privacy) to determine the presence or absence of zebra/quagga mussels throughout their jurisdiction during two times in the mussel's reproductive season. CLI worked with their staff to determine the best times to collect plankton samples and gave them information on high risk areas so they could select the highest risk areas for their sampling scope. CLI also provided them with a sampling protocol and shipping instructions to ensure the best quality sample possible arrived in laboratory. The water samples that CLI did receive indicated that the water sample was taken properly and that calciferous shell organisms were still viable. Our results did not detect the presence of zebra/quagga mussels, and a representative sample of similar organisms found in one of the samples is shown in Figure 1 below.



**Figure 1: Representative FlowCAM Images taken from National Scenic Riverway Sample**

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An example of targeted, time sensitive service comes from a western State Wildlife Department (name withheld for privacy) which CLI assisted in expedited sample analysis for waterbodies that were involved in approved species transfer to another waterbody. The species being transplanted were endangered, so this service was for highly sensitive waterbodies and sometimes the sampled waterbodies were in the proximity of known infested waterbodies. The window for species transfer was typically relatively tight, so lab ability to provide quick turnaround was paramount. The Department notified us of an impending sample and would ask for a one week turnaround. CLI would move their sample(s) to the front of the queue, complete the sample analysis within 3 days, and submit the report within 5 days, weekends included. The sample preparation and analysis followed the exact same standard protocol as our standard testing, with the only changes being an expedited internal handling procedure. Again, our results did not detect the presence of zebra/quagga mussels, and a representative sample of similar organisms found in one of the samples is shown in Figure 2 below.



**Figure 2: FlowCAM Images taken from State Wildlife Department Sample**

CLI has also taken steps to ensure our laboratory procedures meet or exceed industry standards by fine tuning our sample preparation and FlowCAM analysis protocols as well as participating in the Bureau of Reclamation's (BOR) Dreissena Veliger Double-Blind Round Robin Study – Round 2. Some of the steps we've completed internally include:

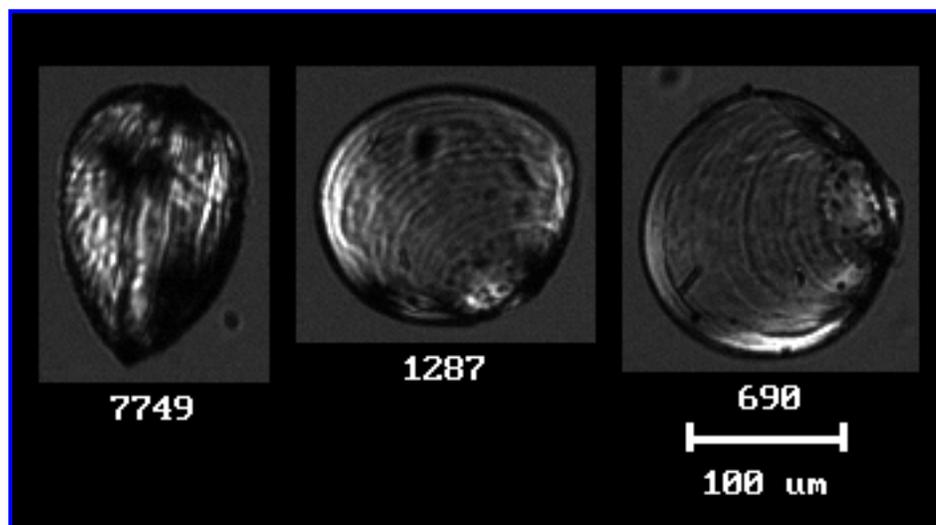
- Updated FlowCAM's Visual Spreadsheet Analysis Software and recalibrated the image sensor.
- Fine-tuned the image sensor capture settings for more vivid birefringence contrast of bivalve calciferous shells.

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- Enhanced sample preparation techniques for maximum organism capture

As noted above, CLI participated in the BOR's Dreissena Veliger Double-Blind Round Robin Study – Round 2 to improve lab accuracy and establish best practices for participating labs. Our lab participated in the BOR study last spring along with 23 other labs intended to determine the relative accuracies associated with FlowCAM Image Flow Cytometry (4 labs), Cross Polarized Light Microscopy (CPLM) (9 labs), and Polymerase Chain Reaction (PCR) (11 labs) veliger detection techniques. The study also required each participating lab to submit their standard protocols for each technique. The results of this study were just released and can be found [HERE](#). The FlowCAM labs didn't perform quite as well as CPLM in this study but did considerably better than PCR. FlowCAM labs posted a 92% accuracy rate (compared to 96% for CPLM) in detection of veligers with half of the labs posting a 100% success rate. The 2 false negatives did occur at the lowest concentration of veligers tested (2-4 veligers/25mL) indicating that there is still work to be done to improve FlowCAM techniques at very low veliger concentrations going forward. However, CLI feels the FlowCAM technology has shown rapid improvement in its ability to detect low concentrations of veligers and refinements of the preparation and testing protocols going forward will continue to close the gap with CPLM while still offering the low labor requirements and autoimage capture which are the hallmark of the technology.

CLI's lab will be working closely with the BOR and the FlowCAM manufacturer Fluid Imaging in this process to ensure that our customers can feel confident that our results meet or exceed the industry standards of CPLM labs. Please see Figure 3 below for images of detected quagga mussels in this study. Note that CLI's lab did not count the center image as a quagga mussel.



**Figure 3: FlowCAM Images taken from BOR Sample**

The BOR also asked participating labs to take a survey about their operations that looked at types of lab techniques offered, days of the week services are offered, turnaround times, expedited services, existing lab certifications, quality assurance procedures, third party QA, willingness to submit to certification for dreissena veliger services, willingness to pay for certification, and others. CLI is pleased that there is a nationwide attempt to standardize dreissena veliger detection lab protocols and provide certification for labs that meet minimum standards. CLI believes this will provide greater confidence among waterbody managers when they are selecting labs to provide dreissena veliger detection services. CLI is equally convinced that the high standards CLI holds our lab to will easily meet and exceed the minimum standards set forth when a certification standard is established. A copy of the study report can be viewed on CLI's web site at <http://www.cleanlake.com/newspublications.html>.

For more information regarding this article, please contact Leif Elgethun whose information is listed below.

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